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In Vitro Activities of T-3262, NY-198, Fleroxacin (AM-833; RO 23-6240), and Other New Quinolone Agents against Clinically Isolated *Chlamydia trachomatis* Strains

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The in vitro activities of three newly developed quinolone drugs (T-3262, NY-198, and fleroxacin [AM-833; RO 23-6240]) against 10 strains of clinically isolated *Chlamydia trachomatis* were assessed and compared with those of other quinolones and minocycline. T-3262 (MIC for 90% of isolates tested, 0.1 μg/ml) was the most active of the quinolones. The NY-198 and fleroxacin MICs for 90% of isolates were 3.13 and 62.5 μg/ml, respectively.

Recently, it has become well known that *Chlamydia trachomatis* is an important human pathogen. It is responsible not only for trachoma but also for sexually transmitted infections, including lymphogranuloma venereum. In women, it causes cervicitis, endometritis, and salpingitis asymptomatically (19), while in men it causes nongonococcal urethritis, postgonococcal urethritis, epididymitis (13), and also probably prostatitis (14). Also, infertility resulting from salpingial obstruction is considered to be frequently caused by *C. trachomatis* (6). Several studies have shown that some kinds of quinolone, macrolide, and tetracycline drugs are effective against *C. trachomatis* in vitro (1, 2, 7, 10, 15–18). We evaluated the in vitro activities of three newly developed quinolone agents, T-3262, NY-198, and fleroxacin (AM-833, RO 23-6240), against 10 clinically isolated *C. trachomatis* strains and compared them with those of other quinolones and minocycline. Three control strains were also tested with the same antimicrobial agents.

McCoy cells were grown in antibiotic-free medium consisting of Eagle minimal essential medium supplemented with 1% glutamine–10% (vol/vol) fetal bovine serum.

Ten clinically isolated strains of *C. trachomatis* were used. All these strains were isolated from male patients who were attending our department. Three laboratory strains (D/UW-3/CX, G/UW-57/CX, and L_2/434/Bu) provided through the courtesy of the National Institute of Health, Tokyo, Japan, were also tested.

The antimicrobial agents used were ciprofloxacin (Bayer Pharmaceuticals), norfloxacin and fleroxacin (Kyorin Pharmaceuticals), enoxacin and pipemidic acid (Dainippon Pharmaceuticals), T-3262 (Toyama Chemical Industry), NY-198 (Hokuriku Pharmaceuticals), and minocycline (Lederle Japan). Stock solutions (1 mg/ml) of quinolones were prepared in sterile distilled water and were maintained at 4°C for a maximum of 2 weeks. Immediately before use, twofold dilutions of the drugs were prepared in maintenance medium containing Eagle minimal essential medium, 1% glutamine, 10% fetal bovine serum, 5.4 g of glucose per liter, and 1 μg of cycloheximide per ml. Minocycline was prepared in the same manner immediately before use.

McCoy cell monolayers were suspended in antibiotic-free medium to a concentration of about 2×10^5 cells per ml. A 1-ml sample of suspension was seeded into flat-bottomed tubes with glass cover slips and incubated at 37°C in 5% CO_2 for 24 h. The monolayer was inoculated with 10^3 inclusion-forming units of *C. trachomatis*. The tubes were centrifuged at 2,000 × *g* at 25°C for 45 min and left undisturbed at room temperature for 2 h. Then the medium was replaced with 2 ml of maintenance medium including twofold dilutions of various antimicrobial agents, and the cultures were incubated at 37°C in 5% CO_2. After 48 h, the glass slips were taken out, fixed with absolute methanol, and stained with Giemsa stain. The formation of inclusion bodies was examined at ×100 and ×400 magnification. The MIC was defined as the lowest concentration of drug which inhibited all inclusion body development in infected monolayers.

Table 1 shows the MICs of various antimicrobial agents for 50 and 90% of the clinically isolated *C. trachomatis* strains (MIC_{50} and MIC_{90}, respectively). Among the new quinolone drugs, T-3262 was the most active antichlamydial agent, with an MIC_{90} of 0.1 μg/ml, followed by ofloxacin (MIC_{90}, 1.56 μg/ml), but it was not so active as minocycline (MIC_{90}, 0.05 μg/ml). The activities of enoxacin and fleroxacin were intermediate, and pipemidic acid was the least active agent. The MICs of each drug for three control strains ranged within those for the clinically isolated ones.

Gross morphological changes in inclusion bodies were observed at a few dilutions below the MIC. Inclusion bodies became fewer, smaller, and pycnotic. Degenerations of McCoy cells were caused by high concentrations of the drugs. Our study confirms the previous reports in which high activities of ciprofloxacin and ofloxacin were evaluated (1, 2, 10, 16, 18). The MICs of pipemidic acid, norfloxacin, and enoxacin were comparable to those found by other investigators (1, 10). The MICs of minocycline were as favorable as those previously reported for doxycycline and tetracycline (2, 10, 16, 18). This study also showed that T-3262 is very effective against *C. trachomatis* in vitro. Recently, the effectiveness of some other quinolone drugs was reported (4, 11, 18), but T-3262 is more effective.

Referring to the clinical efficacy of these drugs against genital chlamydial infection, the trial with norfloxacin and ciprofloxacin had disappointing results (5, 8), while ofloxacin results were encouraging (3, 9). Since T-3262 is more active than ofloxacin in vitro, as mentioned above, and the concentration in serum varies from 0.4 to 0.8 μg/ml when a dose of 150 mg three times daily is administered (unpublished data),

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TABLE 1. Antichlamydial activities of new quinolone antimicrobial agents and minocycline against 10 clinically isolated strains of *C. trachomatis*

Antimicrobial agent	MIC (μg/ml)		
	Range	50%	90%
Pipemidic acid	50–100	50	100
Norfloxacin	12.5–25	12.5	25
Ofloxacin	0.78–1.56	0.78	1.56
Enoxacin	3.13–6.25	6.25	6.25
Ciprofloxacin	0.78–3.13	1.56	3.13
NY-198	1.56–6.25	3.13	3.13
T-3262	0.05–0.2	0.1	0.1
Fleroxacin	3.13–6.25	3.13	6.25
Minocycline	0.025–0.05	0.025	0.05

sufficient distribution of the drug to eradicate *C. trachomatis* is expected.

It is pointed out that about 50% of women and 25% of men with gonorrhea are concomitantly infected with *C. trachomatis* (12). The in vitro activities of T-3262 against *Neisseria gonorrhoeae* have been confirmed (MIC_{90}, 0.0125 μg/ml; unpublished data), and the outcome of a clinical trial was encouraging (12 of 13 patients with positive genital cultures for *N. gonorrhoeae* were freed of the organism after treatment with a single oral dose of 150 mg; unpublished data). Thus, this drug does merit evaluation against concomitant infection with these two organisms.

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